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EDITORIAL

HORSES AND SHOEING

THE summer is at an end, and with its passing, all those glorious occasions in the open air when ordinary people can feast their eyes upon the horse temporarily fade away.

Flat Racing and Steeple-chasing have their devotees but, except for those races which might be described as the great national events, most of the ardent and regular followers of these sports are of a specialised type with an abiding interest in racing economics. The majority of the people exhibit no interest in the beneficial effects that horse racing may have upon the breeding of horses, their support tends from the same sentiments that induce them to fill up their football pool coupons diligently each week. Horse shows and jumping competitions reach the hearts of the millions whose daily life allows of no personal contact with the noble beast.

It is remarkable that, in this highly mechanised "atomic" age, when the machine has driven the working horse from the town and countryside, such deep interest in our old friend still remains, and from Spring to Autumn the horse reigns supreme in all his glory at thousands of shows and exhibitions up and down the country. These shows could not exist if the public did not attend and pay at the gate.

What is the reason for this great interest? Is it evoked by a sense of revulsion against the mechanical devices which have made life full of anxiety? Is it because deep down in our hearts we feel some affinity to other living things,

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or is it that by tradition and breeding we are all horsemen at heart although we may have no opportunity to display a potential prowess of hands and legs? Is it because being instinctively assured that we "know a horse" we can match our opinions, without fear or favour, even from a ring-side seat, against the experts, or is it because the "artist" in us responds to demonstrations of grace and splendour and superb co-ordination between man and beast?

Much can be said in favour of the last argument, for it is a poor soul who cannot delight in the elegance of a lady's hack which preens around the ring with such grace and poise, that one can well imagine that in its passage the blades of grass were not bent, or in the vigorous ebullient hackney which, with animated precision, flashes by with a burr of wheels and to a sound of rustling silk.

All these joys could come to an end if there were no farriers to apply the shoes, just in the same way that modern mechanical transport would be halted in the absence of tyres. Those who function in the ring cannot help but be shocked by the neglected state of many feet and by the quality of the shoeing. The foot is a most important part of any horse. Neglect and incorrect shoeing can ruin the best of feet.

Owners are in a quandary and in most cases the damage that is being done is not of their seeking or fault. There is a great shortage of farriers, be they good or bad, and in an attempt to solve their difficulties many owners are put to the expense of transporting horses for distances of up to twenty miles to get a job done which, previously, could have been accomplished in their own village.

How can the matter be resolved? There is one way. The Veterinary Schools might establish Schools of Farriery on their premises. Courses of instruction could be devised which would include a consideration of the anatomy and physiology of the foot. The metallurgical aspect should include such modern innovations as acetylene welding and wrought iron work, a knowledge of which would be of great economic value to any village smith. A course orientated on a scientific basis need not be as long as the older apprenticeship method in which knowledge, often erroneous, was acquired by example and rote.

In past years the Army School of Farriery was able to turn out an adequate supply of competent men. There is no reason why the same methods cannot be applied in civilian life. A great opportunity to render service to the community awaits those who will grasp it.

GENERAL ARTICLES

OBSERVATIONS AND EXPERIMENTS ON INACTIVATED RINDERPEST TISSUE VACCINES*

By J. K. H. WILDE **

DURING the past fifty years, efforts to control rinderpest resulted in the development of several methods of prophylaxis against the disease. One of these, which for a considerable period served a very valuable purpose, was the outcome of various attempts to attenuate or inactivate the rinderpest virus by chemical means. Kakizaki, *et al.* (1925 and 1926) reported success with the tissues of infected cattle inactivated by the action of glycerine and toluol and other substances, combined with incubation or storage at room temperature. This line was followed by several workers, including Daubney (1928) and Evans and Cornell (1928), and a vaccine which proved valuable for field use was developed. In broad outline, this was produced by pulping certain tissues of cattle reacting to rinderpest and inactivating the virus content by means of formalin. Many variations, both in the tissues used, and in the details of production, have been tried. The definitive method used in Tanganyika depended on the use of spleen and certain lymphatic glands (pre-scapular, pre-crural, popliteal and sub-lumbar) finely pulped and mixed with sterile 50 per cent glycerine in the proportion of one part of pulp to four of glycerine by weight, the virus being inactivated by formalin in a concentration of 1 : 1,000. This type of vaccine has proved of great value in the field control of rinderpest but its use is beset with difficulties, the most important of which are: (1) the possibility of spread of the disease by the infection of vaccinated animals which, according to Hall (1933) and Evans and Cornell (1937), may suffer an inapparent attack of the disease and yet be able to infect susceptible animals; (2) the apparently short duration of the immunity engendered by this type of vaccine in some animals, if not reinforced by subsequent virus infection; (3) the bulk of the vaccine and the high cost of production.

The development of goat attenuated rinderpest virus and later lapinised virus overshadowed the value of inactivated tissue vaccine. Therefore, except for immunisation of very highly susceptible or valuable cattle, this tissue vaccine became virtually obsolete and there was a tendency to abandon efforts to improve it and overcome the objections to its use. However, with the realisation, especially in West Africa, that the goat and rabbit attenuated viruses do not provide an entirely satisfactory solution to the problem of rinderpest control, thoughts are being turned once more to the possibility of producing a more effective inactivated tissue vaccine.

* This paper is from a thesis submitted for the degree of M.Sc., University of London, 1948.

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It may, therefore, be of value to report some of the work and observations carried out by the author in Tanganyika before the production and use of inactivated tissue vaccine were suspended.

Workers on rinderpest have tried various tissues for virus content and for antigenicity when used in the production of inactivated vaccines. Blood, although of high virus content, has been proved valueless as vaccine by Kakizaki (1925) and Daubney (1928). Organs other than spleen and glands, e.g., tonsils, have been shown to have high antigenic value. For many reasons it has proved impracticable to include the smaller of these in vaccines. The larger ones, such as liver and lungs, however, should they prove satisfactory antigenically and in the technique of production, are worthy of consideration as constituents of inactivated tissue vaccine. Contrary opinions have been expressed as to the antigenic value of liver, but Daubney (1928) has shown that, in any case, liver substance is entirely unsatisfactory because it is extremely difficult if not impossible to produce from it a sterile vaccine without using undesirably high concentrations of formalin. The present author considered that lung material was worthy of trial and conducted the following experiments to determine whether this organ could be used in the production of vaccine without reducing appreciably the value of the vaccine while at the same time reducing the cost per dose.

Experiments to Determine the Value of Infected Lung as a Constituent of Inactivated Tissue Vaccine

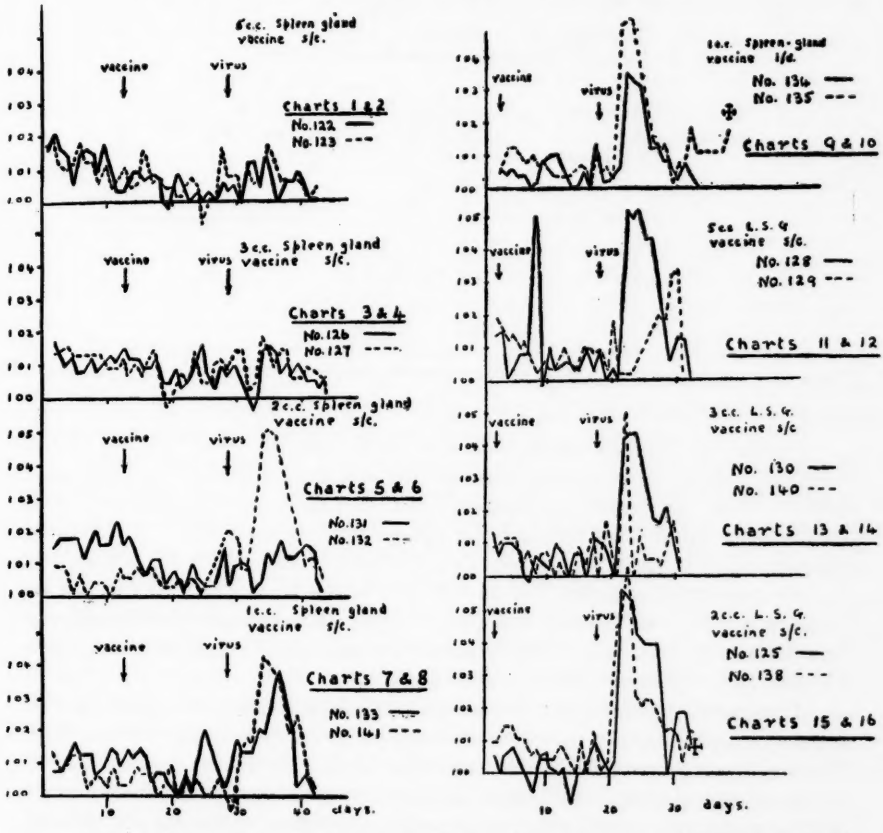
Experiment 1. Preliminary tests showed that, should lung tissue prove satisfactory as an antigen, the average production of vaccine could be raised from approximately 250 to 850 doses per beast.

Three susceptible oxen from a group of vaccine makers which were infected with rinderpest by means of the injection of virulent spleen material, showed typical pyrexia. They were slaughtered on the fifth day after inoculation and their lungs, spleens and certain lymphatic glands were removed aseptically. The lungs were cut into small pieces and minced as finely as possible in a mincing machine. The resulting pulp was mixed with the spleen and gland pulp of the same oxen, and the whole made up as usual with sterile 50 per cent glycerine and 1 : 1,000 formalin. A modification of the routine method had to be introduced in the final stages of the preparation of this brew, as the grinding machine was not capable of breaking down the lung tissue into a sufficiently fine pulp. The consequence was that pulped lung tissue floated on top of the mixture and attempts had to be made to break it down further by vigorous agitation with sterile glass beads. Even then the mixture was very coarse and the pulp had to be passed through wire gauze before it was suitable for injection. Tests were then made with this vaccine in comparison with the routine spleen-gland products.

Four pairs of susceptible oxen were given doses of routine vaccine of 5 c.c., 3 c.c. and 1 c.c. per animal subcutaneously. Another pair received 1 c.c. each intradermally as a result of a suggestion made by Henderson (1945), that

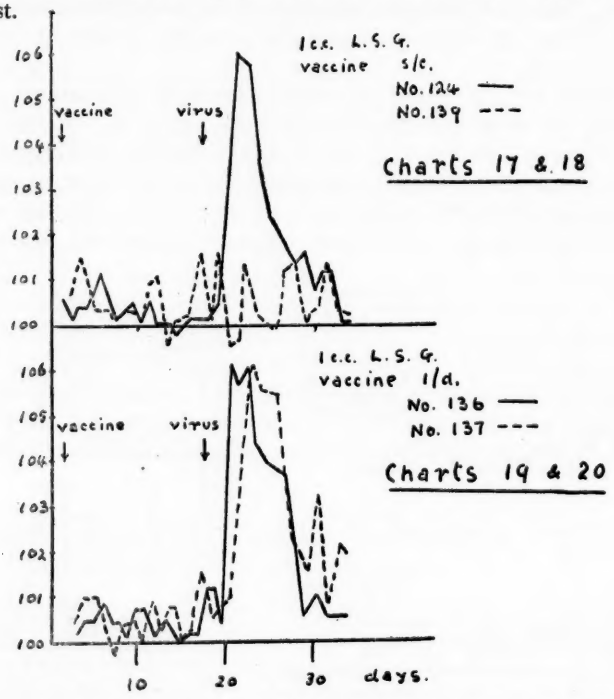
intradermal injection, by bringing the antigen into earlier contact with the recticulo-endothelial system might stimulate a more rapid production of antibodies. Unfortunately, it was impracticable to apply this theory to higher doses. Ten other oxen received doses of the lung-spleen-gland vaccine (referred to hereafter for convenience as L.S.G. vaccine) similar to those given in the former group.

With one exception, no reaction followed vaccination. The exception showed an isolated temperature-peak of 105.2° F. on the fifth day after subcutaneous injection of 5 c.c. of L.S.G. vaccine. This was not typical of rinderpest and as the animal subsequently proved to be susceptible, it is considered that there was no live virus in the vaccine. All these oxen were given a challenge dose of virulent blood subcutaneously sixteen days after vaccination



and the detailed results are shown in Charts Nos. 1 to 20. All those animals which received 5 c.c. and 3 c.c. and one of those which received 2 c.c. of routine spleen-gland vaccine, proved to be immune sixteen days later. All the remainder which received this vaccine reacted to the test although most of

the reactions were mild, showing little beyond pyrexia and temporary malaise, but two were severe, viz., No. 132 which had received 2 c.c. of vaccine and No. 135 which had received 1 c.c. of the vaccine intradermally. This animal died of rinderpest.



Of the ten animals which received L.S.G. vaccine, nine reacted to the challenge dose of virus, although one of the reactions was only transitory and atypical. No. 139, which had received 1 c.c. of L.S.G. vaccine subcutaneously, did not react to the virus test and was presumably immune before vaccination. This was exceptional as all the animals came from a herd which had shown 100 per cent susceptibility in those of its members which had been used for vaccine production. The reactions in this group ranged from mild thermal manifestations (Nos. 129 and 140) to fairly severe thermal and clinical reactions (Nos. 128 and 130) and a severe fatal attack in No. 125.

It appears, therefore, that the animals which received spleen-gland vaccine in doses of 5 c.c. and 3 c.c. received complete protection within sixteen days. This is remarkable considering the small doses used, when earlier workers (Kelser, *et. al.*, 1928; Rodier, 1928; and Kakizaki, *et al.*, 1928) had found much larger doses of inactivated vaccines necessary to produce such strong protection. The larger doses of L.S.G. vaccine gave some measure of protection, but insufficient to prevent manifestations of the disease.

The same L.S.G. vaccine was tested again over a wider range of dosage and the results are shown in Table No. 1.

In this experiment the effect of L.S.G. vaccine was detectable only in the slightly delayed reaction and mildness of symptoms in the animals receiving the larger doses.

TABLE No. 1.

Ox No.	Dose of L.S.G. vaccine in c.cs.	Reaction to virus test after 14 days.	Remarks.
142	8	Thermal only.	Slightly delayed but with peak of 105°F. on 5th & 6th days.
143	6	Thermal with mild cachexia	Similarly delated but peak of 104.4°F. on 6th day.
144	4	Mild thermal with mild cachexia	Similarly delated with peak of 103.4°F. on 5th day.
145	2	Thermal and clinical.	Severe, followed by death.
146	nil	Thermal and clinical.	Severe, followed by death.

Experiment 2. Two more "brews" of L.S.G. vaccine were prepared (designated 3/4 and 17/4). In the preparation the tissue was broken down more thoroughly by double mincing and extra agitation and soaking before sieving. Tests were made on these and the results tabulated (Table No. 2).

These tests indicated a protective property in both "brews." Nos. 171 and 174 were immune when tested and may have been so before vaccination. The antigenic properties of these samples of vaccine were not, however, as pronounced as those of the routine spleen-gland vaccine and "brews" 3/4 and 17/4 could not have been used in the field with safety.

Experiment 3. Two further batches of L.S.G. vaccine, designated 11/5 and 13/5, were prepared, another modification in the technique being introduced. The lung material was minced twice and separately and to it was added only half the usual quantity of 50 per cent glycerine. The spleen-gland portion of the vaccine was made in the usual way and then the two preparations were mixed. These were tested as shown in Table No. 3.

It appeared again that the vaccine had engendered some degree of protection, but this was much too uncertain to allow of the issue of the vaccine for field use.

Experiment 4. The "brew" 13/5 was tested again, alone, and also when mixed in equal proportions with a routine spleen-gland vaccine (1/7). The results are shown in Table No. 4. For comparison in this test, other animals were vaccinated with routine spleen-gland vaccine ("brew" 29/6).

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In the interpretation of these tests, the possibility of prior resistance in some degree cannot be overlooked; examples are Nos. 47 and 53. On the other hand, it is possible that the aberrancies may be explained by envisaging a varying ability

TABLE No.2.

"Brew" 3/4.

Ox No.	Dose of L.S.G. vaccine in c.cs.	Reaction to virus test after 14 days.	Remarks
171	10	No reaction	Immune.
172	8	Thermal only	Reaction typical but recovery rapid.
173	6	Thermal only	Reaction typical but recovery rapid.
174	4	No reaction	Immune.
175	2	Thermal with obvious clinical symptoms.	Recovery slow.
176	nil	Thermal and clinical.	Severe reaction ending fatally.
"Brew" 17/4.			
177	10	Thermal with mild cachexia.	Rapid recovery.
178	8	Thermal with more typical symptoms.	Reaction delayed but symptoms more the usual for a susceptible animal. Slow recovery.
179	6	Thermal and fairly severe clinical reaction.	Typical of mild rinderpest with slow recovery.
180	4	Thermal and mild cachexia.	Rapid recovery.
181	2	Thermal and clinical.	Moderately severe but not fatal.
182	nil	Thermal and clinical.	Severe and fatal.

in the different oxen to respond to antigenic stimulus, the results being manifested because the amounts of vaccine injected were small and too near to the threshold of minimum effectiveness. It does appear however, from these tests, that

TABLE No.3.

"Brew" 11/5.

Ox No.	Dose of L.S.G. vaccine in c.cs.	Reaction to virus test after 29 days.	Remarks.
198	10	Thermal with mild clinical symptoms.	Peak 103.4°F. Rapid recovery.
199	8	Thermal with more severe symptoms.	Peak 105°F. Recovery more protracted.
200	6	Thermal and clinical.	Peak 104.2°F. Symptoms as for No.199. Recovery Slow.
1	nil	Thermal and clinical.	Severe rinderpest but followed by recovery.
"Brew" 13/5.			
2	10	Thermal and clinical.	Peak 105.4°F. Severe rinderpest followed by recovery.
3	8	Thermal and clinical.	Very mild attack with rapid recovery.
4	6	No reaction.	Immune.
5	nil	Thermal and clinical.	Severe fatal rinderpest.

TABLE No.4.

"Brew" 13/5 alone.

Ox No.	Dose of vaccine in c.cs.	Reaction to virus test after 14 days.	Remarks
45	10	No reaction	Immune.
46	6	Thermal and clinical.	Severe rinderpest ending fatally.
47	2	No reaction	Immune.
"Brew" 13/5 mixed with "Brew" 1/7.			
48	10	No reaction	Immune.
49	6	No reaction	Immune.
50	2	Thermal reaction and mild cachexia.	Characteristic of very mild rinderpest followed by recovery.
"Brew" 29/6 Routine spleen-gland.			
51	10	No reaction	Immune.
52	6	Doubtful thermal reaction only.	5th day temperature only. No other symptoms.
53	2	No reaction	Immune.
54	nil	Thermal	) Characteristic thermal reaction; slaughtered for vaccine production.
55	nil	Thermal	)
56	nil	Thermal	)

the higher the proportion of spleen-gland tissue in the mixed vaccines, the higher the antigenic value.

Before final conclusions were drawn on the value of lung tissue in the preparation of inactivated tissue vaccine, a batch of vaccine was prepared carefully from the lungs of an ox slaughtered for routine vaccine production. The proportions used were similar to those of routine production and the vaccine was tested in doses of 10 c.c. and 6 c.c. on four oxen. In no case did it confer an immunity or any noticeable protection.

TABLE No.5.

Ox No.	Dose of vaccine in c.cs.	Reaction to virus test 14 days later.
72	10	Typical rinderpest reaction in all animals, indistinguishable from the reactions in completely susceptible cattle.
73	10	
74	6	
75	6	
76	nil	ditto.
77	nil	

It was concluded therefore, that lung material taken from vaccine producers at the height of their thermal reactions when incorporated in vaccines, acted as little more than a diluent and its use was abandoned.

Summary

1. A brief account of the development of rinderpest tissue vaccine is given.

2. Experiments to determine the value of lung tissue from infected cattle in such an inactivated vaccine are described; it is shown that this tissue is not worthy of inclusion in the vaccine as its antigenic value is too low.

The Effect of Cold Storage on Five Types of Rinderpest Tissue Vaccine

Variable results have been reported on the retention of antigenic properties by various types of inactivated tissue vaccine, during storage. A survey of the literature suggests that these properties and their retention by the vaccine, vary according to the method of preparation, the animals involved and the inactivating or preserving agents used. As the vaccine produced in Tanganyika was of such vital importance in certain outbreaks, tests were made on the storage of the routine vaccine and on vaccines of similar but modified type.

A spleen gland emulsion was prepared and divided into five lots. These

were made up respectively into the following types of vaccine, the dilution being the same in each case (20 per cent):—

(a) Routine formolised glycerine vaccine.
(b) Formolised normal saline vaccine.
(c) Glycerine normal saline vaccine.
(d) Glycerine vaccine.
(e) Normal saline vaccine.

In each type in which formalin was used, the final strength of this agent was 1 : 1,000 and where glycerine was used it was always as a 50 per cent aqueous solution.

The vaccines were stored in a refrigerator at approximately ± 4°C. and tests were made at intervals. At every test, five presumed susceptible oxen were injected subcutaneously with 10 c.c. of each of the vaccines to be tested. Subsequent virus tests comprised the injection of 2 c.c. of freshly virulent blood.

The results are described below:—

1. *After Six Months of Refrigeration.*

(a) *Routine formolised glycerine vaccine.*

None of the oxen reacted to vaccination but, after injection with virus, four showed mild thermal reactions with no clinical symptoms and one proved to be completely immune. This vaccine therefore engendered a high degree of protection after six months storage.

(b) *Formolised normal saline vaccine.*

In all five oxen, erratic high temperatures were shown after vaccination, but these were not considered as due to rinderpest. The virus-test caused characteristic thermal reactions with only mild clinical manifestations in three of the oxen and the other two proved immune. This vaccine therefore gave a fair degree of protection after six months of storage.

(c) *Glycerine normal saline vaccine.*

High thermal reactions characteristic of rinderpest, followed vaccination in three of the vaccinated animals. One of these died of rinderpest and subinoculation from one of the others caused rinderpest in a susceptible ox. The other two oxen which failed to react to the vaccine together with those which recovered from vaccination proved immune to the virus. It is possible that these two animals were immune before vaccination. Some of the virus was therefore alive in this vaccine after six months of refrigeration and was not markedly attenuated.

(d) *Glycerine vaccine.*

There was no thermal reaction to vaccination and three of the oxen proved to be completely immune when tested with virus. The other two showed characteristic thermal reactions but no clinical symptoms. This vaccine was therefore capable of conferring a high degree of protection after six months of storage.

(e) *Normal saline vaccine.*

No reactions followed vaccination and four of the animals were immune at the time of the test with virus. The fifth showed a very mild thermal reaction

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only. The vaccine was therefore of high antigenic value after six months in the refrigerator.

2. *After Twelve Months of Refrigeration.*

(a) *Routine formolised glycerine vaccine.*

None of the animals reacted to vaccination and only one reacted thermally to the injection with virus. This showed no clinical symptoms. The vaccine therefore had retained high antigenicity after twelve months of storage.

(b) *Formolised normal saline vaccine.*

None showed reactions to the vaccine but all reacted after the injection with virus and showed one or more of the following symptoms : nasal discharge, ocular discharge, gum lesions, anorexia and diarrhœa.

This vaccine was not as effective after twelve months of storage as at six, nor was it as effective as the formolised glycerine vaccine. It showed no evidence of containing living virus.

(c) *Glycerine normal saline vaccine.*

None reacted after vaccination but three reacted after virus injection. These three and one other showed symptoms of mild rinderpest and one died after a protracted illness. The fifth was immune.

The vaccine contained no live virus and gave uncertain protection after twelve months of refrigeration.

(d) *Glycerine vaccine.*

None reacted after vaccination. Four gave thermal reactions after virus injection and showed mild symptoms. One was immune to the test.

This vaccine continued to give protection after twelve months of refrigeration.

(e) *Normal saline vaccine.*

None reacted after vaccination but all reacted to injection with virus and showed obvious signs of rinderpest. They all recovered very slowly.

Whatever protection this vaccine gave after twelve months of storage, was inadequate and the vaccine at this stage was considered valueless.

As a control to the virus-tests two susceptible oxen were injected with virus at the same time as were the experimental animals in this series. Both developed typical rinderpest and one died.

3. *After Twenty-one Months of Refrigeration.*

Only two of the original five " brews " were tested, the other three being considered as unworthy of test.

(a) *Routine formolised glycerine vaccine.*

No reactions occurred after vaccination. Three oxen gave temperature reactions only after injection of virus, the remaining two were immune.

(d) *Glycerine vaccine.*

The results were the same as those of (a) above. Both of these " brews " were therefore of good antigenic value even after twenty-one months of refrigeration.

Two control animals injected with virus at the same time as those vaccinated, developed severe rinderpest and died.

Finally, the bottle containing the remnants of the brew (a), formolised glycerine vaccine was removed from the refrigerator after twenty-four months of storage and allowed to stand at room temperature which varied within the limits of 60 and 75°F. This brew was tested each week on a susceptible ox and the results are tabulated in Table No. 6. No further tests were made with this vaccine.

TABLE No.6.

No. of ox.	Interval between removal from refrigerator and use in days.	Remarks Results of virus tests.
82	nil	A mild, almost imperceptible thermal reaction on the 3rd and 4th days and no clinical symptoms.
100	7	A reaction of the 105°F. order commencing on the 3rd day. Defervescence complete by the 8th day. No clinical symptoms other than staring coat.
18	14	Very mild thermal reaction on 3rd, 4th and 5th days. No clinical symptoms.
39	28	A thermal reaction rising from 103.6°F. on the 2nd day to 105.4°F. on the 4th day. Return to normal by the 7th day. No clinical symptoms.
50	35	Thermal reaction attaining 104.6°F on the 3rd day. Normal by the 8th day. No clinical symptoms.
67	42	No reaction. Immune.
74	49	Thermal reaction reaching 104.0°F. on the 3rd day. Return to normal by the 4th day. No clinical symptoms.

It is apparent from this experiment that the routine formolised glycerine vaccine retained strong immunising properties after storage for two years in the refrigerator and subsequently for forty-nine days at room temperature. The glycerine vaccine brew was the only other type which approached the routine formolised glycerine vaccine in efficiency after long refrigeration. There appeared to be little to choose between these two vaccines but as it was felt that a vaccine, in which the virus had been inactivated by means of formalin was safer for use earlier after preparation than one which had not been treated with formalin, it was decided to continue the use of the formolised type.

Summary

1. The potency of five types of rinderpest inactivated tissue vaccine have been compared after storage.

2. The best vaccine contained 50 per cent glycerine and little difference was detectable between the formolised spleen-gland-glycerine vaccine and spleen-gland-glycerine vaccine with no formalin, except that the former became inactivated and therefore safe earlier than the latter.

3. Those vaccines containing normal saline were of markedly poorer keeping quality than those which did not contain saline.

4. The formolised spleen-gland vaccine retained a high degree of potency after two years of storage in the refrigerator and 49 days at ambient temperature subsequent to removal from the refrigerator.

Durability of Immunity Engendered by Inactivated Tissue Vaccine

An injection of inactivated tissue vaccine into a susceptible ox will, if the dose is large enough (5 to 10 c.c.) produce in that animal by the tenth to the fourteenth day, an immunity which will not break down even to large injections of virulent bovine virus. This immunity remains solid for several months, but eventually wanes, the animal becoming completely susceptible again. When this vaccine was first introduced into field use, the temporary nature of the immunity was known and a method of "triple vaccination" was adopted. This meant that the animals to be immunised were given three doses of the vaccine with an interval of a week between doses. It was assumed that the second and third doses enhanced the immunity engendered by the first and would hence, prolong the period of immunity.

The following experiment was made. Fourteen young bullocks drawn from a susceptible herd were inoculated with 10 c.c. of inactivated tissue vaccine. Eight of these received a second similar injection one week later, and of these eight, four received a third injection a week later. These animals were grazed with the susceptible herd and were kept free from any contact with rinderpest. On each occasion that an injection of vaccine was given a dose from the same batch was given to each of two other animals from the susceptible herd. These two were withdrawn from the herd ten days after vaccination and challenged with bovine virus. On each occasion, they proved immune to challenge, demonstrating the normal expected efficacy of the batches of vaccine used.

The vaccinated animals were then challenged at intervals as follows:—

Two animals receiving single vaccination were challenged at five months. No reaction occurred. The remaining two animals which had received single vaccination were challenged at eight months and reacted to the virus giving a very mild clinical reaction. It appeared, therefore, that the immunity engendered by the single dose of vaccine, had by the eighth month, waned to the extent of permitting a mild reaction to the virus to take place. Blood drawn from these two animals on the first and second days respectively after injection of virus, was inoculated into other susceptible cattle. This was done in order to determine whether or not, the virus had established itself in the tissues even had the challenged animals not reacted. As was to be expected, the virus was recovered in this way.

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At ten months, two of the animals receiving double vaccination were challenged. One proved immune and the other gave a mild thermal and clinical reaction. Rinderpest virus was recovered from blood from the animal which reacted to challenge, drawn on the second day after the injection of virus into that animal, but not from that which proved immune.

Five days later, the remaining two double vaccinated animals were challenged. One gave a very mild thermal and clinical reaction, and the other proved immune. Blood transmission experiments showed that the virus had not established itself in the blood of the immune of these two oxen.

The first pair of triple vaccinated animals was challenged at the same time and proved immune (*i.e.*, at ten months). Two and a half months later, the remaining pair were challenged and also proved immune.

On the occasion of every test two animals drawn from the susceptible herd were challenged with the same virus, and in every case these animals reacted typically showing that the virus was viable.

Conclusions

(i) A single 10 c.c. dose of the inactivated tissue vaccine used, was capable of giving a solid immunity by the tenth day. This immunity remained strong until at least five months but had waned to the extent of permitting rinderpest virus to cause a mild reaction by the eighth month.

(ii) A double injection of the virus with a week's interval between the injections, prolonged the solid immunity so that at ten months two animals were still immune but two others showed mild reactions on challenge.

(iii) Triple vaccination at weekly intervals extended the solid immunity up to at least twelve months as none of the triple vaccinated animals supported the virus either at eight or twelve months.

Acknowledgments

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MICROBIOLOGICAL ASPECTS OF THE SWINE ERYSIPELAS ORGANISM, *ERYSIPELOTHRIX RHUSIOPATHIÆ*

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THIS organism is responsible for swine erysipelas, and erysipeloid in man; practically all animals and birds, especially the turkey, may also be infected. Precise figures regarding losses of stock in Great Britain are difficult to obtain, although of the swine diseases erysipelas is considered fourth in importance and its incidence is said to be "of considerable importance but not to the extent of a group where more than 10 per cent of the national herd is estimated to be affected to some extent" (O.E.E.C., 1952).

In Great Britain the disease has been sporadic and has occurred principally in the Eastern Counties (Gledhill, 1948), where it has occasionally led to a high mortality (Doyle, 1953); infection is widespread in Northern Ireland and the incidence has greatly increased in recent years (Lamont, 1953). Turkey flocks have grown greatly since the war and there have been serious outbreaks of *E. rhusiopathiæ* infections, often resulting in heavy financial loss (Dobson, 1953).

Loeffler (1886) gave the first accurate description of the organism as a slender, short or slightly curved rod giving a "test-tube brush" appearance in a gelatin stab culture. Smith (1897) isolated an organism in an outbreak of swine erysipelas which formed tiny, transparent colonies on a solid medium and gave variable fermentation reactions. Subsequently, both the cultural and the biochemical reactions of the organism have been studied considerably.

TABLE I.

Fermentation reactions of E.rhusiopathiae recorded by different authors.

AUTHOR	Glucose	Galactose	Mannose	Laevulose	Arabinose	Xylose	Lactose	Maltose	Sucrose
Schipp	A	O	O	O	O	O	A	O	-
Creech	A	-	O	A	-	-	A	-	-
Zibert	A	A	SA	SA	SA	O	A	A	SA
Meyn	A	A	SA	SA	SA	O	A	A	SA
Stiles et al	A	A	O	A	O	O	A	O	O
Deem et al	A	O	V	A	V	V	A	O	O
Morrill	A	O	O	O	O	O	A	O	A
Rosenwald et al	A	O	O	A	O	O	O	O	O
Barber	A	O	O	A	O	O	A	O	O
Watts	A	O	O	A	V	V	A	A	V
Karlson et al	A	A	A	O	-	-	A	-	-
Atkinson	A	O	O	O	O	O	A	A	-
Topley et al	A	O	O	O	O	O	A	O	O
Bergey	A	A	A	A	-	-	A	-	-
Woodbine(a)	V	V	V	V	-	V	V	-	-
(b)	A	A	O	A	-	O	A	A	-
Sneath et al	A	A	A	A	-	SA	A	-	-
Seaman et al (c)	A	O	O	O	O	O	O	A	O
Marsh	O	A	O	A	A	O	A	-	-
Beaudette et al	O	A	O	A	V	V	A	O	O
Van Roekel et al	O	O	V	O	-	V	O	O	O
Ten Broeck	O	O	O	O	A	-	O	-	-
Giltner	O	O	O	O	O	O	O	A	O
Cornell et al	O	O	O	O	O	O	O	A	O
Wayson	O	O	O	O	O	O	O	A	O

- No reaction
SA-slight acid, A-acid, V-variable, O- none recorded
(a) without serum (b) with 5% serum.

TABLE II. Origin of 44 Strains studied.

1.	K.C.H. 19 Human Skin Biopsy	26.	CN 353 Gledhill
2.	Pilgrim III	27.	CN 354 Antwerp
3.	Cliff skin	28.	CN 445 S.E. 11.
4.	Cliff washings	29.	CN 446 Evans
5.	D	30.	CN 906 Venn.
7.	M.E.W. 22	31.	CN 1061 W.E. 2
8.	E.W. 16	32.	CN 1926 44074 Turkey, Connecticut(U.S.A.) 1942
9.	L 32	33.	CN 1927 H-17 Turkey, New Jersey(U.S.A.) 1946
10.	L 8	34.	CN 1928 87193 Hog, Nebraska(U.S.A.)
11.	E.W. 24	35.	CN 1929 V.R.I Turkey, Massachusetts(U.S.A.) 1944
12.	P.L.A.	36.	CN 2023 Argentina 1947
13.	R.V.	37.	N.U.S.A. 1 New born piglets, Sutton Bonington 1953
14.	Chile	38.	N.C.T.C. 1224 Wragg
15.	=R	39.	" 1694 Aberdeen
16.	R.O.X.	40.	" 2422 Solly
17.	W.W.1	41.	" 6332 Joint ill in lamb
19.	N.C.T.C. 1414	42.	" 6333 Duck
20.	" 2420	43.	" 7999 Rouget virulent
24.	" 3406	44.	" 8163 a.P. 15
25.	" 5926		

TABLE III

Biochemical reactions of Erysipelothrix rhusiopathiae.

Strain	Litmus milk	Nitrate reduction	Fermentation reactions without serum. Glucose	Galactose	Mannose	Laevulose	Arabinose	Xylose
1.	SA	SR	-	SA	-	SA	SA	-
2.	-	SR	-	SA	SA	SA	A	SA
3.	-	-	SA	-	-	SA	SA	-
4.	SA	SR	SA	-	-	SA	SA	-
5.	SA	SR	SA	-	SA	-	-	-
7.	SA	SR	SA	SA	SA	SA	SA	-
8.	SA	-	-	SA	A	-	SA	SA
9.	SA	SR	-	-	-	SA	A	SA
10.	SA	SR	SA	-	SA	SA	SA	-
11.	SA	-	SA	SA	SA	SA	SA	-
12.	SA	-	SA	-	-	-	A	-
13.	SA	-	SA	SA	-	-	SA	SA
14.	-	-	SA	-	SA	SA	-	SA
15.	-	SR	-	-	-	-	SA	-
16.	SA	SR	SA	-	SA	-	-	-
17.	SA	SR	-	SA	SA	SA	-	-
19.	-	SR	SA	-	SA	SA	SA	-
20.	SA	SR	SA	SA	SA	SA	SA	-
24.	-	-	SA	SA	SA	-	SA	SA
25.	SA	SR	SA	-	-	SA	-	-
26.	SA	SR	SA	-	SA	-	SA	-
27.	-	SR	-	SA	-	SA	-	SA
28.	-	SR	SA	SA	SA	SA	-	SA
29.	SA	SR	SA	-	SA	SA	SA	SA
30.	-	SR	SA	-	SA	SA	SA	-
31.	-	SR	SA	SA	-	-	A	-
32.	-	SR	SA	-	-	SA	-	-
33.	SA	SR	SA	-	SA	SA	SA	SA
34.	-	SR	-	-	SA	-	SA	-
35.	-	SR	SA	SA	-	SA	SA	-
36.	-	SR	SA	-	-	-	A	-
37.	-	SR	SA	SA	SA	-	A	-
38.	-	SR	-	-	SA	-	A	SA
39.	-	-	SA	SA	-	-	-	-
40.	SA	SR	-	-	SA	-	-	-
41.	SA	SR	SA	-	SA	SA	SA	-
42.	SA	SR	-	SA	SA	SA	-	-
43.	SA	SR	SA	-	-	SA	A	SA
44.	-	SR	SA	-	SA	SA	SA	SA

- = No reaction SA = Slight acid A = Acid SR = Slight reduction

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A survey of the biochemical reactions recorded over the past fifty years shows only some measure of agreement regarding the fermentation reactions. Reference to Table I gives some indication as to the variations that have been recorded.

A total of 44 strains were made available for study (see Table II) by the courtesy of Dr. A. C. Cunliffe (1), Dr. A. W. Gledhill (2-25, 38-44) and Mr. H. Proom (26-31). The strains numbered 32-35 are of American origin and comprise the strains used by Grey (1947*a*,*b*,*c*) in his experiments on mice and turkeys; they were provided by Dr. L. T. Giltner. Number 36 represents what is believed to be the first strain of *E. rhusiopathiæ* isolated in Argentina (Proom and Harris, 1948). Dr. J. C. Buxton provided 37, which was received on blood agar, but all the other cultures were obtained in a freeze-dried condition. Of the 44 strains, five failed to grow from the freeze-dried state—6, 18, 21, 22 and 23.

The strains were studied for their fermentative ability in different carbohydrates (see Table III). These reactions were all carried out in peptone water containing 1 per cent sugar. After inoculation the tubes were incubated at 37° C. for twelve days, readings being taken at two-day intervals.

All the strains gave a negative acetylmethyl carbinol reaction (Voges Proskauer), and failed to produce ammonia, catalase and indole. The strains did not reduce methylene blue and were negative to methyl red; H_2S was positive for all strains.

Based on the experience of previous workers a further series of experiments was performed using 5 per cent horse serum, and galactose, glucose, mannose and lævulose were fermented consistently. Rhamnose, sucrose, trehalose, raffinose and inulin remained negative as without serum; lactose, maltose, dextrin, glycogen and starch were negative without serum but became acid with serum added; arabinose and xylose, which were variable without serum gave negative results when serum was added. Further fermentation reactions were tested using salicin, æsculin, dulcitol, mannitol, sorbitol, adonitol and inositol. Negative results were obtained for all strains and there was no change in a further test using 5 per cent horse serum. An antiserum prepared in horses against strain 27 agglutinated 1 per cent glucose broth cultures of all strains giving titres of 1 :400 to 1 :1600. Tests were made in a water bath at 55° C.; incubation was continued for four hours and the reactions were read; the tubes allowed to stand over night and read again. The restricted value of the antiserum agglutination test from the veterinary diagnostic point of view does not detract from the value of the test in *in vitro* laboratory work.

A review by Woodbine (1950) dealt with penicillin but there seems to be no definite dosage. This depends on individual assessment of the case and the age of the animal involved; it is proposed to consider here only those antibiotics of more recent introduction.

Streptomycin protected mice provided high doses were used (Woodbine, 1947) but has not met with the same success as penicillin. The bactericidal dose in mice was toxic, although smaller doses were effective. Prier and Alberts (1950)

found that 6.25 to 50 μg. per ml. were needed to inhibit the organism after 96 hours in *in vitro* studies. Streptomycin has, however, been used successfully in the treatment of erysipelas in turkeys by pellet implantation (Eveleth, *et al.*, 1949).

Bacitracin has been used by Mantovani and Maglione (1952) for the treatment of mice infected by *E. rhusiopathiæ* and they found that one dose of 250 units increased the survival time while about 500 units in six doses cured all the infected mice.

Polymyxin has been investigated and is inactive (Stansley and Schlosser, 1947; Woodbine, 1948).

In vitro studies by Prier and Alberts showed 12.5 to 200 μg. per ml. of aureomycin to be necessary to inhibit growth of the organism after 96 hours. They found that 20 mg. given to pigeons at the time of infection and again 24 hours later decreased mortality. Lower doses were not effective, neither were doses of 100 and 200 mg. administered 24 and 48 hours after infection. One dose of 100 mg. or 400 mg. in two intramuscular doses 24 hours apart were not toxic.

TABLE IV Sensitivity of E. rhusiopathiae to terramycin.

Time (hours)	Number of strains	Inhibitory concentration μg per ml
24	3	1.0
	16	0.50
	12	0.25
	5	0.10
	2	0.06
48	14	2.0
	15	1.0
	5	0.50
	4	0.25
72	36	2.0
	1	1.0
	1	0.50
96	All strains(38)	2.0

TABLE V Sensitivity of E.rhusiopathiae to aureomycin

Time (hours)	Number of strains	Inhibitory concentration μg per ml
24	7	2.0
	15	1.0
	7	0.50
	6	0.25
	1	0.10
	2	0.06
48	34	2.0
	1	0.50
	2	0.25
	1	0.10
72	All strains(38)	2.0

TABLE VI Sensitivity of E. rhusiopathiae to bacitracin

Time (hours)	Number of strains	Inhibitory concentration μg per ml
24	26	2.0
	10	1.0
	2	0.50
48	All strains (38)	2.0

Terramycin and penicillin have been used by Schneider, Spencer and Ensminger (1951) in feeding trials on swine infected experimentally with erysipelas but no advantage was found using either antibiotic.

Magnamycin in *in vitro* studies by English, Field, Szendy, Tagliani and Fitts (1952) gave a minimum inhibitory concentration of 0.25 μg. per ml. for *E. rhusiopathiæ*.

Studies have been made and we have reported on those strains (except 39) already referred to, and ten antibiotics, namely: penicillin, streptomycin, dihydrostreptomycin, polymyxin (Aerosporin B), chloromycetin, aureomycin, neomycin, magnamycin, bacitracin and terramycin.

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TABLE VII Activity of magnamycin against E. rhusiopathiae.

Time (hours)	Number of strains	Inhibitory concentration µg per ml.
24	Nil	0.50
	4	0.25
	18	0.10
	12	0.06
	4	0.03
48	3	0.50
	15	0.25
	15	0.10
	5	0.03
72	3	0.50
	30	0.25
	3	0.10
	2	0.06
96	5	0.50
	31	0.25
	2	0.10
96-168	NO CHANGE	

TABLE VIII Activity of penicillin against E. rhusiopathiae

Time (hours)	Number of strains	Inhibitory concentration units per ml.
24	1	0.10
	13	0.06
	11	0.03
	10	0.015
	4	0.0075
48	1	0.10
	17	0.06
	13	0.03
	5	0.015
	2	0.0075
72	12	0.10
	8	0.06
	14	0.03
	4	0.015
96	13	0.10
	8	0.06
	17	0.03
96-168	NO CHANGE	

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Solutions of the antibiotics were prepared in 0.85 per cent saline containing two units (penicillin and bacitracin) or two μg. (the other eight) per ml. of antibiotic. By dilution, solutions containing from 1 unit or 1 μg. per ml. to 0.0037 unit or 0.0037 μg. per ml. were obtained. The effect of the various concentrations was determined at intervals over a period of 7 days and incubation was at 37° C.

We have followed the general assumption that an organism not inhibited by 2 units or 2 μg. per ml. of antibiotic is resistent. After 24 hours there was no inhibition at a concentration of less than 2 μg. per ml. by streptomycin, dihydrostreptomycin, polymyxin, neomycin or chloromycetin.

Tables IV, V and VI show the concentrations of terramycin, aureomycin, and bacitracin causing inhibition. With terramycin at concentrations between 0.06 and 1.00 μg. per ml. no growth was evident after 24 hours incubation (see Tabel IV); growth was visible over the subsequent 3 days. All strains had overcome a concentration of less than 2 μg. per ml. by the fourth day. Aureomycin (see Table V) inhibited 31 strains at a concentration of 0.06 to 1 μg. per

TABLE IX Minimum inhibitory concentrations of magnamycin and penicillin (after 7 days)

Number of strains	magnamycin μg per ml	penicillin units per ml
2	0.50	0.10
2	0.50	0.06
1	0.50	0.03
9	0.25	0.10
6	0.25	0.06
15	0.25	0.03
2	0.10	0.10
1	0.10	0.03

ml. The other seven strains were not sensitive to less than 2 μg. per ml. With aureomycin all strains had overcome a concentration of less than 2 μg. per ml. by the third day. Of these three antibiotics, bacitracin was the least active (see Table VI), only 12 strains were sensitive to less than 2 units per ml. and none to less than 0.5 unit per ml. None of the strains was susceptible to less than 2 units per ml. after two days.

Magnamycin and penicillin gave a prolonged inhibition and Tables VII and VIII show the variation in concentrations capable of affecting the various strains over a period of four days. It can be seen from Table VII that at 24 hours *E. rhusiopathiæ* is inhibited by a range of 0.03 μg. to 0.25 μg. of magnamycin per ml. The effectiveness decreases over the four days. Penicillin behaves similarly but the inhibitory range at 24 hours is 0.0075 unit to 0.1 unit per ml. The effect of both drugs became stable after four days and an incubation period of up to seven days caused no further change. Table IX shows the combined minimum inhibitory concentrations of magnamycin and penicillin after seven

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days. In the case of magnamycin an inhibitory range of 0.1 to 0.5 μg. per ml. was obtained with the greatest number at 0.25 μg. per ml. while penicillin had a range of from 0.03 to 0.1 unit per ml. with the greatest number at 0.03 unit per ml.

Summary

The biochemical and cultural reactions of *E. rhusiopathiæ* have been studied and the production of acid when grown in glucose, galactose, lævulose and lactose media coupled with the negative methyl red, acetylmethyl carbinol, indole and catalase tests are helpful in identification.

At the present time penicillin is the most important antibiotic used in the treatment of the infections caused by *E. rhusiopathiæ.*

Further work into the potential synergic action of some of the antibiotics may prove of value.

No *in vitro* antibacterial activity was shown by streptomycin, dihydrostreptomycin, chloromycetin, polymyxin and neomycin; a decreasing antibacterial activity *in vitro* was shown by terramycin, aureomycin and bacitracin, in a descending order of effectiveness; a continued antibacterial activity *in vitro* was shown over a period of seven days by penicillin and magnamycin, the former being the most satisfactory antibiotic under examination.

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XUM

THE BLOOD PICTURE OF THE COW

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Introduction

THE objects of this survey were to examine the blood picture of the cow in this country in greater detail than that already recorded by Fraser (1930), to show the relationship between one set of measurements and another where this was of interest, to review the work of other authors and to try and set some limits of normality as a basis for clinical hæmatology.

Materials and Methods

Hæmatological Technique.

The technique used for blood examination was, in general, that described by Holman (1950). With the exception of blood films and clotting time, blood mixed with about 2 mg. of sodium oxalate per millilitre was used for all measurements. Hæmoglobin was estimated as alkaline hæmatin in a Lovibond comparator. Wintrobe's tubes were spun for an hour for the packed cell volume. A Piney pipette was used to make a dilution of 1/200 for the red count and another to give 1/20 for the white count; unstained platelets were counted in the preparations used for red counts. For the red count an area of 0.08 sq. mm. was counted in each cell of the hæmatocytometer and the result was accepted if the disagreement between totals was less than 10 per cent; the platelet count was on the same areas but no limit of error was imposed. For the white count an area of 2 sq. mm. was counted in each cell and the result was accepted if the difference between counts was less than 10 per cent. The mean corpuscular diameter was found by projecting eosin-stained blood films on to a ground-glass screen at a magnification of 2,000 and measuring the diameters of 256 corpuscles on each of two blood films. Specific gravity was determined by dropping whole blood into aliquots of two mixtures of chloroform and benzol, one at S.G. 1.0 and the other at S.G. 1.1. Viscosity was estimated from duplicate readings using Denning and Watson tubes. To measure clotting time, capillary tubes, filled with blood, and plugged at one end, were kept partially immersed in water at 37° C. and the protruding end was broken off each half-minute. Fragility was measured from tubes containing concentrations of NaCl varying by 0.025 per cent and the first concentration showing complete hæmolysis after 24 hours was recorded; this unusual time period was chosen as a routine method for dealing with clinical samples as it was found that a small time period was often inconvenient. A minimum of 200 cells were classified for the differential count

Animals.

The investigation was carried out on Ayrshire cattle kept at the A.R.C. field station, Compton. Bulls and bull calves were not included in the survey. Bias was observed for animals under three years old and these were treated separately and form the subject of a second article (Holman, 1955, in Press).

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Recordings.

Statistical methods have shown that a well-chosen sample of about 30 gives a very good indication of the mean and distribution of the population measured. Unfortunately, in veterinary hæmatology it is very hard to know what constitutes a well-chosen sample, and the record by Rusoff and Piercy (1946) that blood values from one well-managed dairy herd can vary significantly from those of another in the same district or from those of the same herd during a different month, shows the existence of important but unknown causes of variation. In view of this, figures based on a survey of three different cows taken each month of the year were discarded and the present paper is based on single samples from 81 cows and includes samples for each month of the year, cows housed or kept out of doors, cows in milk and dry cows. This larger sample, although providing approximately the same average figures, gave definitely larger standard deviations and was considered a more practical assessment for clinical purposes.

The standard deviations used throughout the paper are those of the distribution, and not of the mean. In Table I the actual distribution is recorded within limits set by the standard deviation. For normal curves about two-thirds of the 81 measurements should fall within the limit set by one S.D. each side of the mean, 19 out of 20 within two S.D.s of the mean and 99 out of 100 within $2\frac{1}{2}$ S.D.s. Where any measurement was too large or too small to fit within $2\frac{1}{2}$ S.D.s of the mean the actual value of the sample is recorded under the column headed "Values Excluded." By this method it is possible to see how well the distribution of each constituent agrees with a normal distribution and, when the distribution is by no means normal as, for example, when the S.D. is larger than the average, the table gives some idea of the shape of the curve and offers some figures for comparison. Thus with band forms, recorded as 1.22 ± 3.57 the distribution table gives the following information:

0 to 1 per cent (i.e., 1.22 minus 3.57 to 1.22) band forms were present in 67 of 81 films.

1.5 to 4.5 per cent (1.22 to $1.22 + 3.57 = 4.79$) were present in 10 films.

5 to 8 per cent (4.79 to $4.79 + 3.57 = 8.36$) were present in two films.

And one film had 19 per cent and another 24 per cent of band forms.

The above method was not able to show the curve given by basophils and their distribution is recorded in the text.

Erythrocytic Picture

Results for the erythrocytic picture are recorded in Table I. A full comparison of these results with that of other workers is not possible for some authors have merely recorded an average, or an average with a selected maximum and minimum, others have shown the average with the standard error of the mean (and not of the distribution) without quoting the number of animals sampled, and others have spoilt the value of their sample by including more than one measurement for each animal. In addition, past work is usually incomplete because the authors have selected only one or two of the three essential measurements required for the present classification of anæmia, namely, the hæmoglobin value, the packed cell volume and the erythrocyte count.

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In Table I, therefore, the comparison is limited to the values selected as normal by Wirth (1950) in Austria (tables on pp. 158/159) and Coffin (1953)

TABLE I

The Averages and Distribution of some Morphological Constituents and Physical Properties of Cow's Blood

Constituents	Units	Cows sampled	Average	Standard Deviation	Values Excluded	Distribution table: (2.5 S.D.s)	(2 S.D.s)	(1 S.D.)	Average	(1 S.D.)	(2 S.D.s)	(2.5 S.D.s)	Values Excluded	99% Confidence Level for 2h hrs.	Results Recorded by Barth (Austria)	Results Recorded by Coffin (U.S.A.)
Haemoglobin	g/100ml.	81	11.3	1.49	-	1	1	15	19	38	7	5	3	-	1.46	11.0	8.0 - 15
Packed Cell Volume	per cent.	81	33.7	4.14	-	1	2	8	30	30	7	3	1	47	3.72	34.7	30 - 40
Erythrocyte Count	10^6/c.mm.	81	5.95	0.765	-	1	2	14	18	34	12	2	1	-	.812	6	5.4 - 9.0
Mean Corpuscular Volume	c.μ.	81	57.1	7.27	-	1	1	9	36	21	11	2	2	78	7.27		49.5 - 60.7
Mean Corpuscular Haemoglobin	$\mu\mu$ cent.	81	19.2	2.80	-	1	1	10	34	23	10	2	2	27	3.29	31.7*	14.4 - 18.6
Mean Corpuscular Haemoglobin Concentration	$\mu\mu$ cent.	81	33.7	2.81	-	1	0	6	42	18	7	3	0	41	4.64		32 - 34
Mean Corpuscular Diameter	μ.	34	5.75 ■	0.216	-	-	-	5	13	9	6	1	0	6.3	.303	5.7	
Variation in Standard Deviations of Corpuscular Diameters	μ.	34	0.442 ■	0.044	-	1	0	6	13	11	2	1	1	0.589			
Mean Corpuscular Thickness	μ.	34	2.21 ■		-	-	-	-	-	-	-	-	-	-			
Erythrocyte Fragility (NaCl)	g/100 ml.	81	0.474	0.045	-	1	0	6	26	38	5	4	1	two at 0.6	.031	0.48-0.62	
Specific Gravity, Whole Blood	Aq=1000	81	1052	3.9	-	0	1	6	27	29	6	3	1	-	.005	1052	
Viscosity of Whole Blood	Aq=1	81	6.09	1.21	-	1	-	11	29	29	7	3	1	9.6	1.59	3.98	
Platelet Count	10^3/c.mm	61	350		-	-	-	-	-	-	-	-	-	-		400	
Clotting time	mins.	74	6.0	1.5	-	2	1	5	26	25	5	0	1	10.5‡	2.64	6.01	300 - 800
Leucocyte Count	10^3/c.mm	81	7.03	1.96	-	1	0	3	31	24	9	3	0	13.5	2.18	8.0	4.5 - 13
Differential Count: Bands	per cent.	81	1.22	3.57	6	-	-	8	37	10	2	0	1	19 & 24	4.72		
Poly.	" "	81	29.1	9.15	-	2	0	8	37	20	10	2	1	56	15.8	35.9	30
Lymph.	" "	81	51.4	11.8	-	0	1	17	22	29	12	0	1	81	14.2	53.0	52
Mono.	" "	81	8.32	2.7	-	1	0	6	21	20	9	0	1	(16.5/20.5)‡	4.76	5.0	9
Eos.	" "	81	9.67	11.9	-	-	-	-	52	26	9	1	1	-	6.50	6.0	8
Absolute Counts: Bands	10^3/c.mm	81	85.7	290	-	1	1	1	69	28	8	0	1	1330 & 2205			
Poly.	" "	81	2029	837	-	1	0	5	38	17	9	1	1	6552			
Lymph.	" "	81	3635	1234	-	1	0	11	36	17	9	1	1	(6922/8214)‡			
Mono.	" "	81	565	416	-	-	-	6	32	24	4	0	1	3675*			
Eos.	" "	81	709	599	-	1	-	6	44	21	6	1	1	(2295/2837)‡			

■ = Calculated from the averages given.
1 = Based on 5 samples from each of 6 cows
2 = Clotting times of 12 minutes very occasionally recorded in healthy cows not included in survey groups.
3 = Excluded values: 16.5, 16.5, 17.5, 18.0, 20.5.
4 = Excluded values: 6922, 7898, 7913, 8214.
5 = Excluded values: 2295, 2592, 2837.
6 = Hess method.
7 = Bürker apparatus

in the United States (tables on pp. 156/157), in their textbooks concerned with veterinary hæmatology; and appropriate work by other authors is referred to in the text.

For clinical purposes the most important measurement is the amount of hæmoglobin. The value recorded for this was 11.3 ± 1.5 g/100 ml. This value is in good agreement with that of Allcroft (1941) who provided an average of 11.5 ± 1.7 from a sample of 300 cows of various breeds in England and Wales. It can be seen from the table that my figure for hæmoglobin is less than 3 per cent greater than the figure recorded by Wirth and that it falls practically in the middle of the range given by Coffin. It is also in agreement with the figure of 10.9 recorded by Mullick and Pal (1943) for 32 Hissar cows near Delhi in India. Thus the round figure of 11.0 g/100 ml. would appear to give a good working average for cows of many breeds in many countries.

No previous estimates of packed cell volume for cows in this country are available for comparison but the figure of 33.7 per cent is almost identical with the value of 33.4 recorded by Canham (1930) for Friesland and Ayrshire cows in South Africa. Wirth gave the slightly higher figures of 34.7 and the mid-point of the range given by Coffin is 35. Whitby and Britton (1939) give a correction of 1.09 to compensate for the shrinkage due to the use of 2 mg. of sodium oxalate per ml. of blood which, if applied to my results, brings my average to 36.7 per cent. Canham used one part of 7.5 per cent sodium citrate to nine parts of blood so that his samples would also have been affected by shrinkage. It would seem from these figures that where shrinkage did not occur the round figure of 35 per cent would give a good working average.

The Mean Corpuscular Hæmoglobin Concentration derived from the above 32 to 34 given by Coffin and it is suggested that a working average of 33 per cent would be suitable for general application.

The erythrocyte count averaged 5.97 x 10^6/c.mm. which falls between the figure of 5.6 for shorthorn cows in milk and 6.6 for dry cows, recorded by Fraser. When compared with records of authors of other countries it is almost identical with the figure given by Wirth. Coffin gives a range of from 5.4 to 9 suggesting a higher average, but Burnett (1917) had previously recorded that the average count for America was 6 million and this is supported by Ferguson, Irwin and Beach (1945) and Miller (1934) who both recorded averages of 6.3. There seems little doubt that a working average of 6 million would have wide application.

If a certain concentration of hæmoglobin is present in the blood it could be carried by a large number of small cells or a smaller number of large cells. It has been shown that these alternatives do exist in some domestic animals, including the cow (Holman, 1952) and therefore the standard laid down for the erythrocyte count is not so useful as that for hæmoglobin or packed cell volume for the number of cells will vary among individuals even where the hæmoglobin level in the blood is the same. Thus Mullick and Pal, who measured hæmoglobin but not the packed cell volume, gave a hæmoglobin figure of 10.9 g. only one per cent different from the selected working average, and an erythrocyte count of 8.1 million, while Canham, whose packed cell volume was almost identical with mine, recorded an erythrocyte count of 6.7, a difference of 12 per cent.

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The normal variation in the size of the erythrocyte from animal to animal will affect both the indices that are dependant on the erythrocyte count. These are: the mean corpuscular volume and the mean corpuscular hæmoglobin. This variation must be taken into account when attempting to diagnose microcytic or macrocytic anæmia, and would be expected to cause variations in values among different authors especially where their recorded red counts are unusually high or low, through chance or, perhaps, breed difference. In this investigation the mean corpuscular volume averaged 57.1 c. micra, a value that falls near the middle of Coffin's range of about 50 to 61, and as in the majority of herds the erythrocyte count is near 6 million, a useful working average would be 57 c. micra. In contrast to this Canham with a red count of 6.66 million and an unexceptional packed cell volume, recorded an M.C.V. of only 47 c. micra.

The mean corpuscular hæmoglobin is a poor index for it is influenced both by the wide variation in red count and also by the fact that in many cows believed to be healthy the erythrocytes may not be fully saturated with hæmoglobin. The average of 19.2 micro-micrograms recorded in Table I is too high to fall within Coffin's range of normals. In contrast Mullick and Pal's figure of 13.2, produced from a red count of 8.2 million, is too low to fall within Coffin's range. The round figure of 19 micro-micrograms would agree closely with the average recorded in Table I and with an M.C.H. calculated from the standards of 11 g/100 ml. of hæmoglobin and 6 x 10^6/cm. for the red count.

The mean corpuscular diameter given by Price-Jones curves made on 34 cows was 5.75 $\pm$ 0.216 μ. Fraser, using a micrometer eyepiece, recorded an average of 5.0 with a common range of from 4 to 6 and an extreme range of from 3.6 to 9.6. Blount (1939), using a cover slip technique to lessen corpuscular shrinkage, examined samples from 40 cows and recorded an average of 6.5 μ with a common range of from 5 to 7 and an extreme range of from 4 to 8. Both authors refer to the presence of a small proportion of very large corpuscles which Fraser called macro-normocytes to differentiate them from megalocytes that might occur under pathological conditions.

My average falls between those of Fraser and Blount and the distribution given by the total of 17,408 corpuscles as measured on 68 slides from 34 cows gives the following percentages: 4.5 μ, 1.04; 5 μ, 13.54; 5.5 μ, 36.11; 6 μ, 35.53; 6.5 μ, 10.75; 7 μ, 2.14; 7.5 μ, 0.36; 8 μ, 0.01. If it is necessary to have a distinctive name for the largest type of corpuscle then the term "macro-normocyte" would apply to the 80 corpuscles (0.37 per cent) with diameters of 7.5 or 8 μ for these cells do not appear to fit into what is otherwise an ordinary cocked hat curve.

Comparing these results with those of authors in other countries it can be seen that my figure is in very good agreement with that provided by Wirth. Canham records that cells of 6 μ were frequently seen and that cells of 7 μ were observed but gives no mean or distribution. Wintrobe (1951) quotes Kushner for the range of 5.0 to 5.9 but this includes calves which have a smaller cell size. From these results the round figure of 5.7 μ would appear acceptable for herds in which the erythrocyte count was accepted as 6 million.

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Distribution of Data.

A table, giving individual measurements of packed cell volume, hæmoglobin and erythrocyte count for the 81 cows and one additional cow has been published elsewhere (Holman, 1952) but an indication of the distribution for erythrocyte measurements is recorded in Table I and it can be seen that most distributions give the cocked hat type of curve common in biological measurements. Of these curves the packed cell volume is the most symmetrical and the red counts and hæmoglobin figures are skewed to the right; this bias is reflected as a skew to the left in the indices (M.C.V., M.C.H.C. and M.C.H.). It can be seen that an area of 2.5 standard deviations each side of the mean, which should exclude about one per cent of the population, excludes one figure in nearly all the erythrocyte measurements, and inexplicably, but possibly by chance, this figure appeared as a high value whether the curve was skewed to the left or to the right.

Platelets and Physical Measurements

An average platelet count of 350 x 10^3/c.mm. based on 5 serial counts on 6 cows, was obtained, but the technique was not accurate enough for the samples to give a good distribution and there was large individual fluctuation. The counts ranged from 100 x 10^3/c.mm. to 700 x 10^3/c.mm. Fraser, using a more elaborate method, recorded an average of 510 and a range of 470 to 740 x 10^3/c.mm.

For authors in other countries, Wirth gives an average nearer mine and Coffin gives a range very similar to Fraser's. Results depend in great part on the method used and at the present moment each worker must find his own standard of normality. If the modern development of using a solution of cocaine as a dilutent (Dykes, 1951) passes into general use then perhaps some standard that is generally applicable may be produced, but the technique recorded in this article was not considered accurate enough to be worth including in the main investigation.

Erythrocyte fragility, measured for complete hæmolysis overnight, gave an average of 0.474 per cent NaCl. This figure is below the normal range given by Wirth and above Demmel's figure of 0.42 as quoted by Dukes (1947). It would appear that results were largely dependent on technique and that no working average for general application could be fixed on the information available.

The average specific gravity was identical with the figure of 1052 given by Wirth. Dukes records 1061 for bullocks and 1043 for cows. If one accepts this difference as due to environment and not as sex difference, the mean of these figures agrees with a working average of 1052.

My figure for viscosity does not agree with that given by Wirth and here again the result would appear to depend on the method. The average of 6 minutes for clotting time agrees precisely with that recorded by Wirth for the Burker method and is in good agreement with Dukes's figures of 6½ minutes, also recorded for the Burker method.

Erythrocyte sedimentation rate using Wintrobe's tubes or Westergren tubes was measured on a few cows, but even after 24 hours the fall was very small with

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rates for individual animals varying inexplicably from week to week. As Ferrando (1937) reported that the rate had little significance in the cow and as with human blood the point is made that the sedimentation rate must be measured within a few hours from the withdrawal of the blood, the measurement was not included in the main investigation.

Correlations Between Erythrocytic and Physical Measurements

That some correlation exists between the various measurements on blood is obvious and anæmia has often been diagnosed from a low erythrocytic count and even by a low specific gravity.

TABLE II

Correlation Coefficients for Erythrocytes and Related Measurements Based on Measurements from 81 Cows

	Haemoglobin	Packed cell volume	Specific Gravity	Erythrocyte count	Viscosity
Haemoglobin g. per 100 ml.	-	.758	.581	.450	.492
Packed cell volume per centum	.758	-	.446	.439	.457
Specific gravity	.581	.446	-	.396	.303
Erythrocyte count 10^6 per c.mm.	.450	.439	.396	-	.273
Viscosity (f. Ag. = 1)	.492	.457	.303	.273	-
Average of Column	.570	.525	.431	.389	.381

Significance of "r" with 81 items (Fisher and Yates, 1943): .2565 $p = .02$; .2830 $p = .01$; .3568 $p = .001$.

A measure by which it can be seen how closely these physical measurements influence one another is the coefficient of correlation. With this coefficient the better the agreement between two sets of figures the higher the coefficient, so that with perfect agreement the coefficient is 1.0.

Table 2 records the coefficient of correlation between the various sets of measurements and it can be seen that by far the best agreement is between the hæmoglobin and the packed cell volume, the next is between hæmoglobin and specific gravity, and after that hæmoglobin and viscosity. From the column for hæmoglobin it can be seen that the worst agreement is between hæmoglobin and erythrocyte count. Therefore if anæmia is defined as a lack of hæmoglobin, the least sensitive way of showing this lack is by an erythrocyte count. The poor agreement between the erythrocyte count and the hæmoglobin, as compared with the packed cell volume and hæmoglobin, is at least partly explained by the negative correlation between the size and number of erythrocytes in the cow so that when erythrocytes are few in number they may be large in size.

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Where specific gravity is used to estimate hæmoglobin the regression line obtained can be drawn by plotting the following points.

S.G. 1042 = Hb. 9.06; S.G. 1052 = Hb. 11.30;
and S.G. 1062 = Hb. 13.54.

Fragility did not correlate with the other measurements. Wirth quotes Kreuzer (p. 54) as recording a quicker clotting time where viscosity was high and Peterson (1952) has shown a correlation between plasma viscosity and fibrinogen, but in this survey there was no correlation between the viscosity of whole blood and clotting time. Irwin and Bell (1938) suggested a correlation between the erythrocyte count and the white count, a reasonable assumption as both would vary with the water content of the blood, but in this survey there was no appreciable correlation between the 81 pairs of measurements.

Leucocytic Picture

The appearance of the different types of leucocytes has already been described by Fraser and by Blount in this country and by Canham in South Africa. Fraser also pointed out that in cattle a distinction must be made between metamyelocytes (band forms and young forms) and unsegmented polymorphs (the poly. 1 of the Cooke and Ponder count); without this distinction the hæmogram of the cow and most other types of domestic animal loses its value when used to estimate a neutrophilic reaction.

The distribution of band forms given in Table 1 shows that they are limited to one per cent or less in the large majority of films. The two cows showing counts of 19 and 24 per cent appeared in good health and all their other blood measurements fell well within the normal ranges; one had calved a week previously. These two extreme counts must be considered very abnormal and in routine clinical hæmatology their occurrence would suggest a grave prognosis; that such a shift to the left can occur in apparently healthy cattle is a warning against relying too much on a neutro-philic shift to the left by itself.

Fraser does not record his neutrophilic metamyelocytes as a percentage of the differential count but records them as a percentage of the total neutrophils, viz.—

0.1 young forms, 3.5 band forms and 96.3 mature cells. For the Cooke and Ponder count he recorded Poly. 1, 73; Poly. 2, 24; and Poly. 3, 3 per cent. Blount recorded 1 or 2 per cent band forms with less than one per cent in Poly. 4; he also reported that the only polymorph he had seen with five lobes was in a film from a calf.

Basophils averaged 0.37 per cent in this investigation but the distribution was so skewed that neither the S.D. nor the distribution table in Table 1 was adequate for its display, and the distribution is recorded here. With 200 cells classified on 81 films, the distribution was as follows:—

No basophils in 47 films, one in 19 films, two in 7 films, three in 6 films, four in one film and five in one film.

The average leucocyte count in Table 1 agrees with Fraser's figure of 7.63 x 10^3/c.mm. but is well below Wirth's figure of 8.0. Canham records the results of

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twelve different workers, most samples were very small, but the average leucocyte count ranged from 5,486 to 9,730 per c.mm. In view of this variability a standard working average would not be of much practical use.

Owing to the sampling error inherent in the differential count the most stable constituent should be the one that approaches 50 per cent. From the distribution recorded in Table 1 it can be seen that this is the case and that the lymphocytes are well grouped round the average. This average of 51 per cent lymphocytes is identical with that recorded by Fraser and sufficiently near those of Wirth and Coffin (53 and 52 per cent) to allow a working mean of 52 per cent to be of value as a standard. Next in proportion is the neutrophil for which the average of 30 per cent is in good agreement with Fraser's figure of 31 and is identical with that given by Coffin; Wirth, however, gives the higher figure of 36. From these figures a working figure of 30 might be acceptable.

Averages for monocytes and eosinophils agree quite well with Fraser's figures of 7 per cent and 11 per cent respectively. Results from other countries recorded in Table 1 suggest that averages for these cells vary considerably. The average for the four different authors given in the previous paragraph gives 7 per cent monocytes. Thus a good standard differential count would be:—

lymphocytes 52, neutrophils 30, monocytes 7, and eosinophils 11.

Correlations Between Cells of the Differential Count

The two largest classes of cells, neutrophils and lymphocytes, showed an obvious negative correlation. There was also a highly significant ($p = .001$) negative correlation between lymphocytes and eosinophils, suggesting that Seyle's stress reaction, as shown by a decrease in both lymphocytes and eosinophils, had little importance in normal physiological reactions.

No other correlation between different cells was discovered nor was there any suggestion in a dot diagram that the ordinary neutrophilic reaction, combining an increase in neutrophils with a decrease in eosinophils, had introduced bias.

Variations

Age.

The various changes associated with age are recorded in a succeeding paper (Holman, in Press).

Sex.

In this investigation the blood picture of the bull has been ignored because the bulls at this field station were kept under different environmental conditions from the cows and it is illogical to compare cows living under one set of conditions with bulls living under other conditions and then attribute the differences to the sex of the animals, although comparisons of this type can be found in the literature.

Since it was first shown that men had a higher hæmoglobin value than women many workers have reported the same sexual difference in animals, but the conditions of their measurements are hard to find. Canham gives his complete tables comparing a group of males with a group of females under 14 months of

age and another comparison between 14 months and three years. These averages show no difference between the sexes. Fraser showed higher figures for 5 stall-fed bulls compared with 5 stall-fed cows, but also showed low figures for young bulls at pasture, which he attributed to nutrition in one place in his article and to age in another.

Wirth states that the erythrocyte and hæmoglobin figures for male animals are higher but that there are exceptions. Wintrobe (1951) states that the difference in the human male and female is not manifest until puberty and in mammals in which menstrual loss does not occur the values are the same for both sexes. In view of this statement it seems probable that the higher figures for the erythrocytic picture of the male, where present, are due to environment.

To record the changes occurring at parturition, 6 cows were observed and a total of 31 samples was examined. Changes varied in amount in the individual cow but the general results were as follows. From about 12 hours before calving to about 12 hours after calving the packed cell volume had increased from 34 per cent to 40 or 45 per cent. The red count rose equally but the hæmoglobin in one cow rose less definitely owing to a slight hypochromia which was apparent for about a week after calving.

The leucocytic changes consisted of an increase in total count which rose from an average of about 6,000 to about 9,000 per c.mm. some 12 hours before calving. None of the six cows had a true leucocytosis and the highest count was only 10,600 per c.mm. This increase was produced in all cows by an absolute neutrophilia in which the neutrophils more than doubled their previous numbers and showed a slight shift to the left as shown by the presence of about 5 per cent band forms in the differential count. The neutrophilia persisted for about 24 hours and then subsided, but the shift to the left persisted for about three days after calving, except in one cow in which it became pronounced and was still evident a week after calving.

Some 12 hours after calving lymphocytes fell to less than 3,000 per c.mm., this fall was much less noticeable than the fall then apparent in neutrophils and had disappeared the following day. Eosinophils were counted as part of the differential count and this method did not detect any significant change. Monocytes and basophils fluctuated without similarity between cows.

In three of the six cows the clotting time decreased by one or two minutes for one or two days after calving, but this may have been fortuitous, and no significant changes were apparent in the viscosity or fragility. The specific gravity varied with the hæmoglobin.

Many of these changes have already been recorded, thus, Canham recorded rather higher erythrocyte counts about the time of calving and Ferguson, Irwin and Beach (1941) recorded an increase in red count immediately after calving followed by a fall over three weeks to below pre-calving levels. They also recorded a decrease in leucocytes after calving but, being more interested in the statistical variation of averages than in the hæmatology of individual cows, they could calculate no significant changes in either neutrophils or lymphocytes. This is unfortunate for their graphs both for healthy cows and for those infected with

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Brucella abortus show a definite change over in the proportions of neutrophils and lymphocytes on the day after calving. If I interpret the figures correctly, Morris (1944) also shows this change over in the numbers of neutrophils and lymphocytes still apparent in a sample obtained 36 hours after calving in a cow which was being sampled each week. Kerr, McGirr and Robertson (1949) showed the increase in leucocyte count due to an absolute neutrophilia at the time of calving but were more interested in the fall in lymphocytes which they demonstrated in their cow at 6 hours after calving. These authors showed that the fall was similar in timing and extent to the fall produced by the injection of an adrenal-cortical extract.

Ferguson *et al.* (1941) reported a fall in eosinophils and a rise in monocytes over a period of 10 days after calving, but although some fall in eosinophils would be expected to occur during the stress reaction indicated by the fall in lymphocytes it was not possible to confirm this from the differential counts on my cows. They also recorded greater fluctuation in both total and differential leucocytic counts during parturition. No work on physical properties has been noticed except that Wirth records that Machts found no alteration in the erythrocyte sedimentation rate during parturition.

Neither Canham, Morris nor Ferguson *et al.* have recorded any definite changes during the state of pregnancy but the graphs given by these authors do show that from time to time there is a reversal in the ratios of lymphocytes to neutrophils which is not in any way connected with parturition.

No study was made on cows in œstrus, but from the work of other authors the leucocytic picture appears to be similar to that of parturition in displaying a stress reaction. Canham showed no regular rise or fall in the erythrocyte or leucocyte count, but his lymphocyte percentages on the day of œstrum were low and were succeeded by higher percentages the next day. Moberg (1953) confirmed this fall in lymphocytes and by counting eosinophils in a hæmocytometer was able to show a definite fall on the day of œstrus and the succeeding day.

Breed.

The close similarity of the results of this investigation to the standards laid down by Wirth and by American workers suggests that there is little variation among European breeds as recorded in Europe and in the temperate zones of America. A group of workers who suggest breed differences among these breeds are Rusoff, Johnston and Branton (1954), who recorded a significant difference in the packed cell volume dependent on multiple samples from 5 Jersey, 5 Guernsey and 5 Holstein bulls. A breed difference should, however, depend on the distributions given by samples from a large number of animals, and not on a large number of samples from a small number of animals, for this operation could often show significant differences between groups of animals of the same breed.

Among African breeds Canham showed a close similarity between Veld cows and Friesland or Ayrshire cows in the same district. Among Indian breeds Mullick and Pal (1943), recording results for the Hissar breed, report an average red count of 8.2 million for 32 cows and a hæmoglobin figure of 10.9 g. It must be pointed out, however, that this hæmoglobin figure is smaller than that recorded

in their younger cattle and that it may denote a mild degree of anæmia in the adult, nevertheless it is clear that there are a large number of erythrocytes in this breed and it is probable that this is compensated for by the corpuscles being of a smaller size. The average leucocyte count for this sample was 9.1 thousand, a figure in agreement with European cows. Breed difference, when apparent, might therefore be limited to erythrocyte size.

Environment.

Environment undoubtedly plays a big part in causing the differences that are found among groups of cattle of the same breed, but the analysis is made difficult by the wealth of hypotheses based on insufficient evidence. For example, Fraser contrasted the low red count of 5.6 million in four yearling bulls at grass with a count of 10.7 million in bulls four or five years old and attributed this increase to the greater age of the older bulls; this is in contradiction to the theory that the red count falls with age. Canham, on the other hand, discovered a decrease in red count with increasing age and, under the influence of Neser's findings on horses (Neser, 1923), attributed this fall to the fact that cattle take less and less exercise during the first two years of life; he avoided any reference to the packed cell volume which should have shown that the bulk of erythrocytes had not been increased. Much other work is of a similar type depending on a few isolated observations.

Manresa, Reyes, Gomez, Zialcita and Falcon (1940) have recorded that owing to the tropical conditions in the Philippines, Holsteins and Herefords have hæmoglobin values as low as 6.8 g. compared with 9.4 g. for the native cattle, and Manresa and Orig (1941) reported a double cycle in hæmoglobin during each year; the levels rose during the cooler periods and were independent of the values for relative humidity or the leucocyte count. Garner and Unsworth (1953) in Nigeria, show a definite fall in the hæmoglobin values of Zebu bulls, during June and July of each year, which is suggestive of anæmia due to a nutritional defect.

Canham compared the red count of three cows living at a level of 3,500 feet in Natal with three cows under similar conditions and of the same age, living at 4,400 ft. in Transvaal. He found that in each pair the red count was one or two million higher in the higher altitude; the packed cell volumes are not recorded. Similarly, Wirth quotes Clawson as recording that when a group of 27 cows were moved from 5,400 feet to 9,000 feet the red count increased from 8.75 to 9.26 million.

It is obvious that there is a great variation in the erythrocytic picture of the cow due to its environment. These variations have, often by surmise, been attributed to many different factors. If the confusion that now exists is to be cleared away the greatest care must be taken in formulating the logic of an observation before assigning the variation to any one cause.

It has also been found that in dealing with small groups of experimental cows one must be watchful of differences produced in the blood picture by unknown environmental changes, because in many cases all the members of the group are affected so that the changes are assumed to be due to an experimental

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procedure; this fallacy can only be guarded against by having control animals present in the same cow-shed or field so that the fluctuation occurring from unknown causes can be separated from those produced by the experimental interference.

Individual.

When working on the hæmatology of domestic animals one is impressed by the amount of variation shown in the individual animal. This variation may be due to a readily available reservoir of cells (such as the spleen) or to quick alterations in water balance. Neser favoured the first view and demonstrated that the red count of the horse could be greatly increased within a minute or two if the horse was threatened or excited. Fiennes, on the other hand, believed the changes were due to continual changes in the water content of the blood. He stated that with the increase in water in the plasma the erythrocyte increases in size by osmosis so that the packed cell volume tends to remain constant (Twistleton-Wykeham-Fiennes, 1952 *a* and *b*). Fiennes also stated that even though the blood became diluted so that the red count fell, the hæmoglobin value remained constant, this is almost equivalent to saying that the corpuscle is continually losing or gaining hæmoglobin. Fiennes admits that this view is heretical and, as the only data he presented in its support was five serial examinations on one animal, it is not likely to be accepted until further evidence is submitted.

In an attempt to measure the amount of variation to be expected between samples obtained on succeeding days, 50 cows were bled on two consecutive mornings. The differences between the two samples were squared and the standard deviation was obtained from the sum of these squares. Twice this standard deviation is recorded in Table 1 as the " 95 per cent confidence level for 24 hours." This means that if the variation between the first and second day for each constituent was due to numerous unknown causes so that the effect was one of chance then nineteen times out of twenty the difference between the two daily readings would be less than the figure recorded in the table.

Of this daily variation a large part is made up of the error of the method of estimation and is thus unrelated to the animal; the remainder of the variation depends on individual variation and environmental factors and these two together sometimes give a greater variation than if distributed by chance. These confidence levels are therefore not mathematically accurate but they form some guide to the amount of variation that can be expected, and variation which is not outside these limits should not be considered as necessarily due to experimental interference. Thus, if the hæmoglobin value of a cow is 11.3 g. just before experimental interference and the next day the value has fallen to 10.0 g. this difference is less than 1.46 g. quoted in the table and there is no justification for assuming that the treatment has had any effect even if a fall in hæmoglobin was expected. These levels, therefore, when used with the techniques described, act as a safeguard against a too hasty assumption that experimental effects have been produced. It can be seen from Table 1 that these levels are somewhat similar in extent to the standard deviation of the distribution.

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Clinical Standards

Few authors have attempted to lay down standards dividing healthy from unhealthy animals. Of these some have laid down limits of 10 per cent each side of the mean, Coffin has given a normal range in round figures and Holman (1950) has suggested that figures three standard deviations from the mean are definitely pathological. Wirth avoids the subject altogether.

The difficulty in setting standards is complicated by the different standards required by a group or by an individual animal. Thus, if the average hæmoglobin is accepted as 11 g./100 ml. and a group of cows each have a value between 9 and 10 g. then although no individual cow is outside the normal range the group as a whole can be considered anæmic, in that better environmental conditions particularly as regards diet, would be expected to raise the values to nearer the accepted average. This brings us to the question of the definition of anæmia. If an apparently healthy cow has a low hæmoglobin level which could be raised by improving the diet, is it anæmic, or should the term anæmia be reserved for the animal which has such a low hæmoglobin that its health is impaired? From the literature it would appear that the first case if often spoken of as a "physiological" anæmia and the second as a "pathological" anæmia. This same problem occurs with other constituents and it would seem that although such things as low leucocyte values can only be demonstrated in a herd result by statistical tests, it should be possible to lay down some standard for the pathological state of leucopenia in the individual cow which, although not accurate, would at least be a guide to the clinical worker, and that as knowledge increased this standard could be modified by future workers to a more critical value.

It has been shown that for European breeds living in temperate zones there is a good agreement upon the average figures for the main constituents and commencing with the erythrocytic picture we have seen that a round figure of 11 g./100 ml. is acceptable for hæmoglobin. Inspection shows that the lowest figure for hæmoglobin recorded for the 300 cows examined by Allcroft and for the 81 cows examined in this investigation, was, in both cases, 8 g. and this was also the figure given as the end of the normal range by Coffin. There is therefore good justification for taking 8 g. as the end of the normal range and recording figures under this as showing some degree of pathological anæmia.

If the conditions in which the hæmoglobin value is reduced to about half the normal value is regarded as moderate anæmia, then with this as a guide, anæmia can be classified as: Mild pathological anæmia, 7 g.; moderate anæmia, 5 to 6 g.; and severe anæmia, 4 g. and below. By accepting this standard, however, we are specifying that the European breeds living in the Philippines with an average recorded by Manresa *et al.* (1940) as 6.8 g. and the Kumaoni bullocks at Izatnagar in India, with an average of 7.4 g., recorded by Murty and Kehar (1952), are, on the whole, suffering from a mild pathological anæmia. The classification of an anæmia as euchromic or hypochromic depends on the value recorded for the M.C.H.C. The only comparison available is the normal of

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32 to 34 per cent given by Coffin. This is a very restricted range compared to the distribution for the 81 cows recorded in this investigation and my lowest figure after correction for shrinkage is 24.6. On this ground I would suggest 24 per cent as the end of the normal range. Garner and Unsworth describing a seasonal anæmia in Zebu bulls in Nigeria show what is, by our previous standard, a moderate pathological anæmia with the M.C.H.C. falling to about 22 per cent.

Compared with the figures for anæmia in man, our standard would be: slight hypochromia present at 23 per cent becoming moderate at about 18 per cent and severe at 14 per cent. By these standards for M.C.H.C. the Kumaoni bullocks at Izatnagar in India with figures ranging from 19 to 25.3 per cent show individuals with mild hypochromia.

The classification of bovine anæmia by the size of corpuscle is not easy. The size of the corpuscle varies diurnally in the same cow with the amount of water in the plasma (Fiennes), corpuscular size varies from cow to cow, and group differences in size are often marked even in breeds where larger samples give an average red count of 6×10^6/c.mm. and we have noted that results for Hissar cows suggest that some breeds may have a smaller corpuscle.

The limit set by my data are such that a value of 45 c.μ. or more would indicate a macrocytosis. In contrast Coffin's range (49.5 to 60.7 c.μ.) would exclude more than 40 per cent of my samples. Canham's samples ranged from 41 c.μ. to 59 c.μ. with an average closely similar to mine but his findings need a correction for shrinkage as his citrate was over double the concentration now acceptable. With three such different ranges it is not possible to lay down a standard and the confusion is emphasised by Garner and Unsworth who describe a hypochromic anæmia with an M.C.V. value as low as 39 or 40 c.μ. and draw a tentative conclusion that it is a macrocytic anæmia when many values lie below all the three normal ranges referred to.

Blood concentration is best measured by the packed cell volume. For this measurement my data give three samples at 42 and one at 47 per cent. Canham gives two samples at 41 and one at 43 per cent, and Coffin gives a normal range of from 30 to 40 per cent. After correction for shrinkage the limit for Canham's figures and mine are 47 and 51 per cent and these results are sufficiently in agreement to suggest that when shrinkage has been avoided a packed cell volume of 50 per cent or over, can be accepted as evidence of anhydræmia. This standard would suggest the presence of mild anhydræmia in one of my samples.

The criterion of an hypoplastic anæmia is the absence of regenerative changes in the erythrocytic picture. Although a single erythroblast is occasionally seen in the blood of a healthy cow, regenerative changes are not expected to occur until the red count has fallen to about half the average for the species. Fortunately there are two good records of changes following withdrawal of blood from a cow. The first, given by Burnett, is a table recorded by Smith in which blood was withdrawn each day until on the fifth day the red count had fallen to 3 million. On this day the first signs were noted and consisted of the appearance of about 10 per

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cent large erythrocytes—presumably Fraser's macro-normocytes—and 3 per cent of corpuscles showing punctate basophilia. During recovery the main changes seen were the presence of macro-normocytes and punctate basophilia, although an occasional erythroblast was observed.

The second account, given by Wirth, records that when the erythrocytic count had been reduced to under 4×10^6/c.mm. the changes included the presence of reticulocytes, polychromatic cells, punctate basophilia and erythroblasts. He does not comment on the presence of large erythrocytes and the most persistent reaction was the continued presence of Howell-Jolly bodies.

For the total leucocyte count of cows Coffin gives a good normal range of from 4.5 to 13.0×10^3/c.mm. This range covers all Fraser's samples from cows and all but four of my samples. My samples falling under this range were 3.7, 3.8 and 4.2 thousand and these figures together with other unpublished data suggest that the limit of 4.5 is rather high and that only counts of below 4 thousand should be accepted as indicating leucopenia. With this criterion two of my samples show mild leucopenia but in each case the succeeding or preceding count was above 4 thousand and therefore from the clinical point of view the error is negligible. One of Fraser's yearling bulls shows a count of 3,000 but this is likely to be an actual leucopenia as it is one of a group that he records may be suffering from poor nutrition and which seem to be suffering as a group from a slight myeloid hypoplasia. A marked leucopenia as seen over a short period in experimental mastitis (Holman, 1954) appears to be a fall to about 2 thousand but the count can fall to as low as a thousand with a return to normality within a few hours. On the other hand where pre-experimental counts are high, treatment or infections commonly producing leucopenia, may only cause a fall in leucocyte count to 5 or 6 thousand and represent what could be termed a relative rather than an actual leucopenia.

For the higher limit Coffin's figure of 13 thousand can be accepted as the limit for normal range and counts above this can be accepted as indicating leucocytosis. By this criterion none of Fraser's cows, and only one of mine, shows a leucocytosis. It must be remembered, however, that leucocytosis can be physiological as well as pathological. In pathological leucocytosis Arthur (1946) records counts in traumatic pericarditis of over 20 thousand and in one case 30 thousand, this last figure can be accepted as indicating severe leucocytosis.

The leucocytic picture is best interpreted by a study of the trends seen in a series of samples. Isolated pictures judged by mathematical standards are of little value. With neutrophils and lymphocytes it can be taken as a rough guide that if in cattle over 2 years old the usual balance in which lymphocytes are in the majority is upset, then some abnormality is present and the total count can be used to discover if the condition is due to neutrophilia or lymphopenia.

The distribution of neutrophil metamyelocytes in Table 1 shows that band forms are usually absent or exist at less than 2 per cent of the total count. With my data a mild shift to the left would be present when band forms number about 5 per cent of the count, in a moderate shift there would be about equal numbers

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of metamyelocytes and mature neutrophils and in a severe shift there would be about twice as many (sometimes four times as many) metamyelocytes as adult cells. In the cow the grave prognosis associated with a severe shift to the left in many other species is not always justified and very occasionally a severe shift may occur and subside in a few days without obvious change in health status.

Eosinophils vary too much from cow to cow for any standard to be of much help, but where no eosinophils are recorded in a differential count of 200 cells then an eosinopenia can be recorded. Where an eosinopenia accompanies a neutrophilic reaction it may only be exhibited as a fall from a high eosinophil count to a low one and where a fall in eosinophils accompanies a stress reaction it is often concealed by the normal variation in the differential count, and to demonstrate the decrease it would obviously be necessary to use a separate technique and count these cells in a hæmocytometer.

Summary

The averages and distributions of the morphological constituents and physical properties of blood given by samples from 81 Ayrshire cows are recorded in a table.

After comparison with the work of other authors the following figures are put forward as good working averages for several European breeds of cattle living in temperate zones of Europe and America: Hb. 11 g./100 ml.; P.C.V. 35 per cent; M.C.H.C. 33 per cent; R.C. 6 x 10^6/c.mm.; S.G. (whole blood) 1052; M.C.V. 57 c. μ.; M.C. Diameter 5.7 μ.; Coagulation time 6 minutes; Percentage proportions of leucocytes: neutrophils 30, lymphocytes 52, monocytes 7, and eosinophils 11.

Correlations between erythrocytic measurements are given as a table and the results suggest that where hæmoglobin estimation cannot be made the packed cell volume or specific gravity estimations give a better estimate of anæmia than the erythrocyte count.

Differences in blood picture between the sexes are considered unproved but a stress reaction is apparent at parturition and œstrus. Variation due to breed and environment is discussed and some indication of individual variation is included in a table.

The following arbitrary clinical standards are suggested for European breeds over two years old and living in temperate climates: Pathological anæmia is indicated by hæmoglobin values below 8 g./100 ml.; 7 g. denotes a mild anæmia, 5 or 6 g. a moderate anæmia and 4 g. a severe anæmia. Hypochromia is indicated where the M.C.H.C. is below 24 per cent; a figure of 18 per cent indicates moderate hypochromia and 14 per cent severe hypochromia. Ranges for corpuscular size vary among authors and do not permit precise criteria for microcytosis or macrocytosis. Anhydræmia is indicated by P.C.V. values of over 50 per cent.

A leucocytosis (either pathological or physiological) is indicated by leucocyte counts over 13 x 10^3/c.mm. and a severe leucocytosis can amount to

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30 x 10^3/c.mm.; leucopenia is indicated by counts below 4 x 10^3/c.mm.; moderate leucopenia is indicated by counts of 2 x 10^3/c.mm. and in severe leucopenia counts as low as 1 x 10^3/c.mm. are seen. Some other clinical abnormalities are discussed in the text.

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GASTRIC ULCERS IN ADULT CATTLE

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ACCORDING to the standard textbooks, gastric ulcers in cattle appear to be of little importance from the clinical point of view. They are found occasionally at post-mortem examination and most frequently in calves.

In 1952 we encountered three cases of perforating gastric ulcers in adult cattle. Clinical symptoms were exhibited and the lesions were noted at post-mortem examination.

The first two cases occurred in a pedigree Guernsey herd and within a period of six months. The food and methods of husbandry over the previous twelve months were investigated, but nothing unusual was noted. No chemical tests were made as only samples of the food being fed at the time of illness were available. This food appeared to be wholesome and, as was the case during the previous twelve months, had been fed also to the rest of the herd.

We record the histories of the three cases we have observed.

CASE 1.—GUERNSEY BULL, 4 YEARS.

History.—This animal was reported as being off its food and depressed. During the five days under observation the temperature fluctuated between 101.5° and 104° F. When the temperature was high the bull showed a dejected appearance with a lowered head and increased costal respiratory rate. The respirations were shallow but no pain was detected on palpation of the abdomen. Procaine penicillin was prescribed, commencing with 3,000,000 units and followed by half this amount daily. This may have accounted in part for the fluctuating temperature as at first the response appeared to be good. Later the temperature rose to 104.5° and did not fall. Death occurred during the night of the fourth day.

Post-mortem Findings.—A fibrinous peritonitis with an inflammatory reaction extending over the omentum which was located mainly in the region of the abomasum. Intestinal contents were free in the abdominal cavity. A small perforation about 1 cm. in diameter was found in the greater curvature of the abomasum. There appeared to be no macroscopic inflammatory reaction on the peritoneal side of the lesion but the mucous membrane of the abomasum was congested for about half an inch around the perforation. No other ulcers were found and the edges of the perforation were smooth and clear-cut.

CASE 2.—GUERNSEY COW HEIFER, 3 YEARS.

It was reported that this animal had not been doing well for some weeks. It had not been seen by us. The symptoms were mild and ill-defined; at no time did the animal develop acute symptoms.

The cow died suddenly one morning. Post-mortem examination revealed a picture similar to that seen in the bull. There was a fibrinous peritonitis. Three ulcers were found in the abomasum (see photograph), one of which showed a minute central perforation. This material was sent to Mr. E. Cotchin, of the

Ulcer in abomasum of three-year-old Guernsey cow heifer.

(*Article by Tutt and Jull, page* 458)

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Royal Veterinary College, London, who reported as follows and provided the excellent photograph.

"The largest of the three ulcers in the abomasum of the Guernsey cow, received 15th April, shown in the accompanying photograph, was about 7 cm. long and 1.5 cm. wide; the floor was flat, with a central minute perforation, the edges overhanging. Two small ulcers, 5 and 9 cm. away from one end of this large ulcer, were rounded and about 1 cm. across. In section, the ulceration occupies largely the mucosa and part of the muscle coat. Many mononuclear cells are present and fibrosis is seen. Granulation tissue is present on the serous coat. The superficial part of the floor of the ulcer is necrotic and in the necrotic tissue a number of organisms can be seen in H.E. sections, some of which look like *F. necrophorus*.

"This seems to be a chronic gastric ulcer and the appearance suggests that it is a peptic ulcer, probably secondary to some traumatic damage to the mucosa, and that the bacteria are not the primary cause. It has been present for some time. The exact cause of the lesion is not apparent.

"The death of a bull with similar lesions is most interesting and suggests a common primary cause, but I do not think the ulcer here has a specific structure that would indicate what that cause was."

Case 3.—Pedigree Jersey Cow.

History.—This cow calved three weeks before the onset of symptoms. We were told that the cow had been off her food and showing a reduced milk yield for a week prior to our first visit. On the day advice was requested she had worsened considerably. The temperature at midday was 102.5°.

Examination.—The cow was recumbent and could rise only with difficulty. It stood with a dejected appearance and there was a marked arching of the back. She could not be made to depress her back and all her movements were unsteady. The fæces passed were normal. A faint smell of acetone could be detected in the breath. The temperature was 105° and there was costal breathing. With the possibility of traumatic peritonitis in mind the metal detector was used, with negative results. However, electricians had been working in the byre and the possibility that some copper wire had been eaten could not be ignored. A tentative diagnosis of peritonitis, possibly due to a penetrating body of non-ferrous material, was made. It was decided to wait until the following day and review the case again. In the meanwhile sulphamezathine powder was administered. The cow died during the night. Blood smears were negative for anthrax.

Post-mortem.—An extensive peritonitis with an inflamed omentum was found. There was no inflammation of the alimentary tract except on the serosa where it was in contact with the abdominal wall. The reaction was patchy. The abomasum showed a small perforation on its greater curvature. The stoma was 1 cm. in size and was midway between the omasal and pyloric orifices. There was no inflammation of the serosal aspect but the mucous membrane was congested for an area of about three quarters of an inch around the perforation. The edges of the perforation were clear-cut. Three inches away from the perforation there was another area of inflammation, in the centre of which was

a shallow ulcer, about 1 cm. in diameter, lined by partly necrotic material. The ulcer appeared to be similar to a *Fusiformis necrophorus* lesion.

Discussion

Although there is no real evidence, it would appear that the lesion starts as an injury to the mucous membrane of the abomasum, possibly occasioned by a foreign body, which later becomes the site of a focal infection which may prove to be associated with *Fusiformis necrophorus*.

We think that clinical symptoms are manifested only when perforation is imminent. After perforation the temperature rises, there is marked shock and depression increased costal breathing and death within 24 hours.

As regards differential diagnosis we feel that traumatic peritonitis in its early stages resembles the condition closely. At the present time we do not suggest that there is a satisfactory line of treatment as the perforation will have occurred before professional advice is sought. In the earlier stages there would be little or no symptoms other than those of an indefinite digestive disturbance. We think that when symptoms of an acute peritonitis occur and the metal detector gives a negative result the possibility of a perforating gastric ulcer must be borne in mind.

NEWS

THE Animal Health Trust recently announced that Mr. Kenneth C. Sellers, Ph.D., B.Sc., M.R.C.V.S., has been appointed director of their new Farm Livestock Research Station at Stock in Essex. Mr. Sellers is 39 years of age and has obtained distinction on the staff of the Institute of Animal Pathology at Cambridge and more recently as Veterinary Investigation Officer for the Ministry of Agriculture, in Yorkshire. The new station at Stock replaces Houghton Grange in Huntingdonshire and pending completion of the new premises temporary accommodation has been found so that research is being continued without interruption.

NOTICES

THE Distillers Company (Biochemicals) Ltd., on behalf of its distributors, announces that as from September 5 prices have been reduced for the following preparations: Penicillin G, Distaquaine G, Distaquaine Fortified, Distaquaine Suspension, Distavone, Streptomycin (sulphate), Dihydrostreptomycin (sulphate), Mixtamycin, Streptaquaine and Distivit. Full particulars of the alterations in prices may be obtained from the manufacturers.

THE firm of Glaxo Laboratories Ltd. have reduced their prices for the following preparations: Dimycin Solution, Veterinary Mylipen, Streptovex, Streptopen Uterine Tablets, Penicillin Granules. New prices have been operative from September 5 last.

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